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Directionality in the mechanical response to substrate vibration in a treehopper (Hemiptera: Membracidae: *Umbonia crassicornis*)

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Abstract The use of substrate vibrations in communication and predator-prey interactions is widespread in arthropods. In many contexts, localization of the vibration source plays an important role. For small species on solid substrates, time and amplitude differences between receptors in different legs may be extremely small, and the mechanisms of vibration localization are unclear. Here we ask whether directional information is contained in the mechanical response of an insect's body to substrate vibration. Our study species was a membracid treehopper (*Umbonia crassicornis*) that communicates using bending waves in plant stems. We used a bending-wave simulator that allows precise control of the frequency, intensity and direction of the vibrational stimulus. With laser-Doppler vibrometry, we measured points on the substrate and on the insect's thorax and middle leg. Transfer functions showing the response of the body relative to the substrate revealed resonance at lower frequencies and attenuation at higher frequencies. There were two modes of vibration along the body's long axis, a translational and a rotational mode. Furthermore, the transfer functions measured on the body differed substantially depending on whether the stimulus originated in front of or behind the insect. Directional information is thus available in the mechanical response of the body of these insects to substrate vibration. These results suggest a vibration localization mechanism that could function at very small spatial scales.

Key words Substrate vibration · Bending waves · Localization · Treehopper, *Umbonia crassicornis*

Introduction

For many arthropods, vibrations traveling through the substrate are important in communication (reviewed in Markl 1983; Claridge 1985; Gogala 1985; Henry 1994; Stewart 1997; Barth 1998) and in the detection of predators and prey (Brownell 1977; Barth et al. 1988; Pfannenstiel et al. 1995; Meyhöfer et al. 1997). In order to find potential mates, capture prey, or avoid predators, it often will be necessary to locate the vibration source. Localization was once thought not to play an important role in vibration perception, either because vibrational interactions take place at close range (Schwartzkopff 1974) or because the high conduction velocity of vibrational waves will minimize directional cues based on arrival time differences between receptors (see Brownell 1977; Cokl et al. 1985). However, a number of arthropods have been shown to accurately localize a vibration source. Four localization tasks have been investigated, including direction on a two-dimensional surface (Murphey 1971; Wiese 1974; Brownell and Farley 1979; Hergenröder and Barth 1983; Abbot and Stewart 1993; Goulson et al. 1994); direction in the web of orb-weaving spiders (Klärner and Barth 1982; Landolfa and Barth 1996; Barth 1998); direction in one dimension along a plant stem (Cokl et al. 1985); and choice between two stems at a branching point (Latimer and Schatral 1983; Steidl and Kalmring 1989; Ota and Cokl 1991; Roces et al. 1993; Pfannenstiel et al. 1995). Some wave types in substrates including sand (Aicher and Tautz 1990), water (Wiese 1974), and plant stems (Michelsen et al. 1982) have relatively low conduction velocities. For arthropods with a spatial array of vibration receptors (primarily in the legs) in contact with the substrate, the resulting differences in arrival time at different receptors have proved sufficient to resolve the direction of wave propagation (Murphey 1971; Wiese

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1974; Brownell and Farley 1979; Hergenröder and Barth 1983; see also Aicher and Tautz 1990). In some cases, directional cues may also be gained from amplitude differences along a gradient (Rupprecht 1968; Brownell and Farley 1979; Latimer and Schatral 1983; Hergenröder and Barth 1983; Cokl et al. 1985).

Mechanisms of localization in small species on solid substrates, for which the time and amplitude differences between receptors may be extremely small, have been less well studied. The results from studies of large species of scorpions, spiders and locusts may not translate directly to smaller scales. For example, when desert scorpions (with legs spanning 5 cm) were placed on substrates in which conduction velocities were five or more times higher than in their normal sand substrate, their responses were no longer directional, apparently due to insufficient time delays (Brownell and Farley 1979). Similarly short delays would be experienced by an animal for which the distances between receptors were five or more times smaller. The size range of species that use vibration in communication or prey location spans more than two orders of magnitude, from spiders with legs spanning 10 cm (Barth 1993) to whiteflies (Kanmiya 1996) and parasitoid wasps (Sokolowski and Turlings 1987) with legs separated by less than a millimeter.

Here we investigate the possibility of a directional mechanism that does not depend on large amplitude and time differences among receptors. Our starting point is an analogy with an acoustic sound localization system. In the parasitoid fly *Ormia ochracea* (Diptera: Tachinidae), the interaction of two modes of vibration in the mechanically coupled ears generates a directional mechanical response to airborne sound, in spite of extremely small time and amplitude differences between the two ears (Miles et al. 1995). Given a system (i.e., some structure with a mechanical response to propagating waves) with two modes, one mode that responds to the spatial gradient of the quantity being detected and one that responds to the spatial average of the signal over the region sampled, the mechanical response of the system can differ significantly depending on the direction of propagation of the signal. The two modes can combine to convert a small phase difference across the region into a large amplitude difference at various points on the structure (Gerzon 1994). If there are two or more modes of vibration in the oscillation of an insect's body resting on its legs, then the mechanical response of the body to substrate vibration may differ depending on the direction of wave propagation. Studies of two arthropods suggest that the body does constitute a resonant system when driven by substrate vibration (Aicher et al. 1983; Dierkes and Barth 1995; but see Dambach 1972; Rohrseitz and Kilpinen 1997). Indeed, from a mechanical standpoint, an insect can be viewed as a mass (body) on a set of springs (legs), where the response properties depend both on the mass and on the stiffness and damping of the springs (Tieu 1996). If the response is directional, this might provide an additional source of

information that could be used in locating a vibration source. However, we know of no studies that have investigated this possibility.

In this study we ask whether the mechanical response of an insect's body depends on the direction of wave propagation in the substrate. The study species is a membracid treehopper (*Umbonia crassicornis*) that communicates using plant-borne vibrations (Cocroft 1996, 1999). We first determine that *U. crassicornis* are using bending waves in communication, as has been concluded for other insects (Michelsen et al. 1982). We then describe a simulator (a short segment of dowel driven with two piezoelectric actuators. Details of this simulator will be presented in a forthcoming paper (R.N. Miles et al., in preparation) that mimics the properties of bending waves in a stem, allowing precise control of the frequency, intensity, and direction of the vibrational stimulus. With a free-standing insect on the simulator, we use laser vibrometry to characterize the response of the body, especially the thorax, to random vibrations originating from in front of and behind the insect. We find resonance at lower frequencies, two modes of vibration, and a pronounced directionality in the mechanical response to substrate vibration. These results raise the possibility of a directional mechanism in insect vibration perception that could amplify the effects of very small time differences between inputs.

Materials and methods

Study species

U. crassicornis is a subsocial treehopper (Hemiptera: Membracidae) that feeds on the sap of trees and shrubs in the mimosoid Leguminosae. Two features of its biology make it a useful species for this study. First is its relatively sedentary behavior: individuals spend most of their lives on host plant stems, leaving only to disperse to a new stem or plant after reaching reproductive maturity or, in the case of males, to search for mates (Wood 1983; Wood and Dowell 1985; R.B. Cocroft, unpublished data). Correspondingly, adults often will remain stationary for an extended period when placed in a new location, allowing for repeated measurements on an intact, free-standing insect. Second is the central importance of vibrational communication in their biology. Substrate-borne signals are important in communication within broods of young, in maternal care, and in mate attraction and location (Cocroft 1996, 1999; R.B. unpublished data).

Adult *U. crassicornis* were collected near Miami, Florida, USA and maintained in the lab on potted host plants (Leguminosae: Mimosoideae: *Albizia julibrissin*). We restricted our measurements to females, both because females tended to remain stationary longer than males on the stem simulator and because they have been a primary focus of studies of vibrational communication in *U. crassicornis* (Cocroft 1996, 1999). For this study we used females that were approximately 14–21 days old, as judged by cuticle hardness and color. Females had a length (from front to back tip of pronotum) of 9.0 ± 0.5 mm ($n = 35$) and a mass of 48.9 ± 9.2 mg ($n = 32$).

Nature of the vibrational signals

Three studies have shown that bending waves are transmitted along plant stems when the stem is mechanically vibrated in a plane

perpendicular to its long axis (Michelsen et al. 1982; Keuper and Kühne 1983; Barth 1998). Evidence comes both from the magnitude of the propagation velocities measured, which agreed with expected velocities based on properties of the stem, and from the relationship of velocity to frequency (the propagation velocity of bending waves is dispersive, increasing with the square root of frequency; Fung 1965). By extension, the signals of insects that vibrate the stem are also concluded to be transmitted in the form of bending waves, and one study (Keuper and Kühne 1983) documented changes with distance in the vibratory signals of *Tettigonia cantans* (Orthoptera: Tettigoniidae) that show evidence of dispersive propagation. Barth (1998) reports propagation velocities typical of bending waves not just in the stems (pseudostems) of banana plants, but also in their leaves (and in the leaves of *Agave* plants; Barth 1993). Results of two other studies are consistent with the observation of bending waves in plant stems. Rupprecht (1968) measured the propagation of mechanically induced signals along plant stems. He measured both longitudinal waves and what he suggested were transverse waves. However, Michelsen et al. (1982) interpret his measurements of the latter as evidence of bending waves. McVean and Field (1996) report results consistent with the interpretation that bending waves are produced by sharp impact on a tree trunk.

We wished to determine whether the vibrational signals of *U. crassicornis* are transmitted along host plant stems as bending waves. Our approach was to measure the propagation velocity of signals produced by the insects, and ask whether the measured values were proportional to the square root of frequency, as expected for bending waves (Fung 1965). We used two identical accelerometers: Knowles BU 1771, mass 280 mg without the cable, frequency response flat (± 3 dB) from 20 Hz to 5 kHz. The accelerometers were attached with wax 5 cm apart to the underside of a horizontal stem of a 1-m-tall potted hostplant (Leguminosae: Mimosoideae; *Calliandra haematocephala*). The stem had clusters of leaves about every 5 cm, a diameter of 4 mm midway between the accelerometers, and a mass (without the leaves) of 172 mg cm^{-1} . Any mass loading of the stem can change its properties, and thus the ideal method would be to use two lasers; however, this was not possible for our measurements. Two accelerometers constitute a mass equivalent to an additional 3 cm of stem, and will undoubtedly have an effect on the stem's response properties. Although use of smaller accelerometers would be preferable from the mass-loading standpoint, we found that the trade-off between accelerometer size and sensitivity rendered smaller units unsuitable for the relatively low-amplitude insect signals. However, a plant stem is a heterogeneous structure with branching points, leaves, and changes in diameter, and the effects of adding a small mass to a stem are likely to be within the range of effects of natural variations in stem morphology. In fact, previous estimates of the effect of attaching one 280-mg accelerometer to a similarly sized stem showed a negligible effect on the stem's frequency response in the frequency range of interest (R.B. Cocroft, unpublished data). The accelerometer signals were amplified with custom-built operational amplifiers and digitized at a rate of 12.8 kHz using SIGLAB Model 50-21 (DSP Technology, Fremont, Calif.). Before attaching the accelerometers to the plant, we compared their output by mounting them side by side on an electrodynamic shaker (Brüel & Kjaer model 4809). We drove the shaker with sine waves swept from 50–4000 Hz and confirmed that the relationship between stimulus phase and output phase was identical for the two transducers.

To obtain recordings of vibrational signals, we placed female *U. crassicornis* individually on the upper surface of the stem, about 2 cm outside the two-accelerator array. Some mature females will produce short, broad-band signals after being picked up and replaced on a stem (R.B. Cocroft, unpublished data). Signals detected by the accelerometers were recorded using SIGLAB. For five signals from each female, we calculated a transfer function between the signals from the near and far accelerometers. The phase propagation velocity (c_B) of waves transmitted along the stem was calculated as:

$$c_B = (2\pi f * d)/p \quad (1)$$

where f is frequency in Hertz, d is the distance in meters between the accelerometers, and p is the unwrapped phase difference in radians measured between the signal recorded at the near and far accelerometers (see Cremer and Heckl 1988).

Simulating bending waves

Our initial design for examining the influence of direction on the biomechanical response to substrate vibration was to use a natural stem, with drivers some distance apart and an insect on the stem between them. However, because of the unpredictable filtering properties of natural stems (Michelsen et al. 1982), this method did not yield sufficient control over the stimulus at the position of the insect. Obtaining data with stimuli from each direction would require either identical sources and propagation paths in front of and behind the insect, or the ability to remove the insect, turn it 180° , and replace it in the same location and posture on the stem between two stimulus presentations. We expected that either of these approaches would introduce too many unknowns and subsequent scatter in our results. We therefore created a simulator that mimicked the properties of bending waves in a stem, in which the propagation direction could be switched electronically between measurements. This allowed us to obtain data on an insect having an identical configuration for the stimuli from either direction.

The simulator (Fig. 1) consisted of a short segment of dowel (3.0 cm long, 0.6 cm diameter) and two piezoceramic actuators. The actuators were attached with epoxy at each end of the dowel, parallel to each other and at right angles to the dowel's long axis. The free ends of the actuators were mounted with epoxy on a fixed metal plate.

The actuators were driven with two random noise signals (maximum frequency 7 kHz). The relationship between slope and displacement expected from bending waves in an ideal beam was achieved midway along the dowel by adjusting the phase relationships between the signals at the two actuators. The design of



Fig. 1 Bending wave simulator consisting of a section of wooden dowel (length 3.0 cm) driven by two piezoelectric actuators. A female *Umbonia crassicornis* is at the center of the dowel. The laser was positioned approximately 20 cm opposite the center of the dowel, and thus measured motion in the plane of the long axis of the actuators and the dorso-ventral axis of the insect

this simulator, and the relevant background theory, are discussed in detail in a forthcoming paper (R.N. Miles et al., in preparation). The simulator provided a close approximation of the behavior of bending waves (see Fig. 2), especially in the center of the dowel where the insects' legs were positioned and for the relatively low frequencies used in communication. Although in real stems there will be a curvature of the surface as the wave propagates (Fung 1965), the short dowel segment we used remains essentially straight. However, over the short distance spanned by the insect's legs (about 5 mm), the difference between real and simulated bending wave motion is minimal (Fig. 2). We measured frequencies from 150 Hz to 4000 Hz, spanning the range of frequencies where most of the energy is concentrated in the signals of *U. crassicornis* (Cocroft 1999). Although there is some energy in the insect signals below 150 Hz, we did not obtain reliable data in those frequencies. Figure 3 shows the phase propagation velocity measured with the laser between two points on the dowel (just in front of and just behind an insect placed on the dowel; $n = 5$ insects). The insect will encounter arrival time differences, virtually identical to those on a stem with propagating bending waves.

We used laser-Doppler vibrometry (Polytec OFV 3000 Controller and OFV 302 sensor head) to make non-invasive measurements of the insect and substrate. The laser beam was parallel to the plane of motion of the simulator, with the lens approximately 20 cm directly in front of the center of the vertical dowel

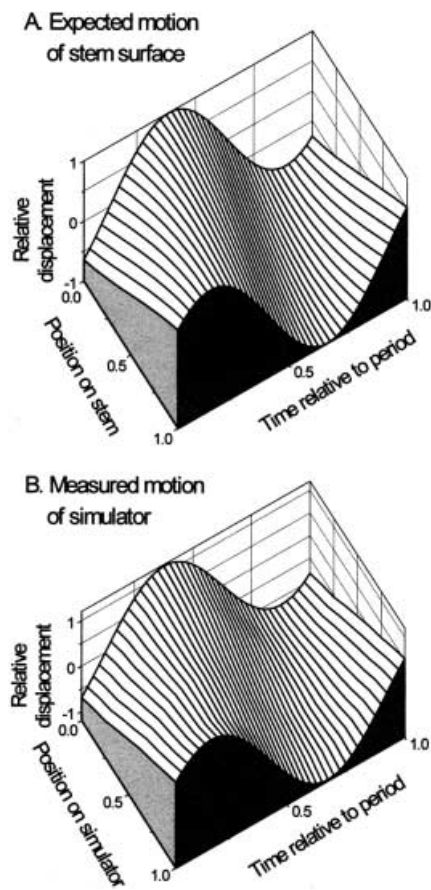


Fig. 2A, B Motion of the surface of a beam during one period of a 500-Hz bending wave propagating along the beam. **A** Motion expected on an ideal beam. **B** Motion on the bending wave simulator, measured with laser-Doppler vibrometry at nine points along the dowel segment. The slope and displacement of points along the surface are almost indistinguishable between the ideal beam and the simulator, except that the surface of the ideal beam shows a slight curvature absent on the dowel

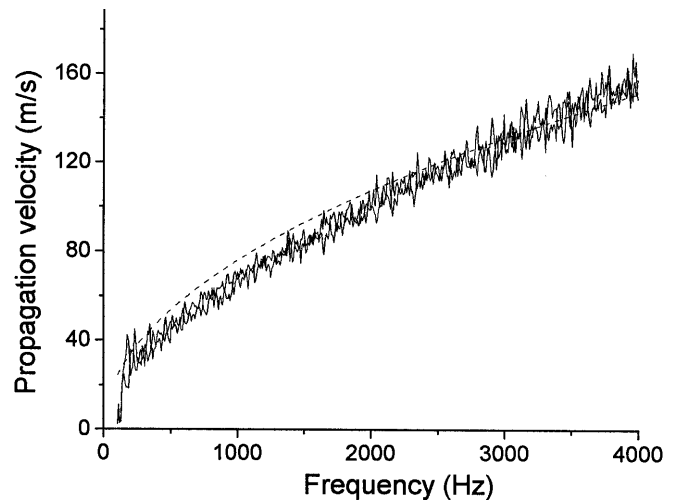


Fig. 3 Mean phase propagation velocity of simulated bending waves, measured with the laser between two points on the dowel (just in front of and just behind an insect positioned in the center). The two *solid lines* represent velocity measured with the signal propagating in opposite directions. The *dotted line* shows the relationship between velocity and frequency that the simulator was designed to reproduce ($2.4 \times$ the square root of frequency; this relationship was estimated from propagation measurements in a plant stem)

segment. It thus measured motion parallel to the long axis of the actuators, and in the dorso-ventral plane of the insects. Fine positioning of the laser beam was done using a two-dimensional motor control unit (Oriol Motor Mike 18000, translators 16627 and 16647). The laser measured velocity with a sensitivity of $1 \text{ mm s}^{-1} \text{ V}^{-1}$ and a maximum frequency of 20 kHz. To increase reflectance of the laser we marked the insects with small dots of reflective paint.

The output signals were generated using a Microstar DAP 2400/6 12-bit data-acquisition PC board. A d.c. bias was added to the output signals so that they could be used to drive the piezoceramic actuators. The output signals were then low-pass filtered at 6 kHz (Frequency Devices 9002 filter, 48 dB/octave), and amplified with a Techron 5515 power amplifier. The laser output was band-pass filtered between 100 Hz and 6000 Hz (Krohn-Hite 3550 filter, 24 dB/octave), and sampled using an Analogic Fast-16 data-acquisition board at a rate of 16 kHz. This filtering provided high coherence between the (a.c.-coupled) output signal and the signal measured by the laser (coherence is a measure of data quality; the coherence function will be unity if there are no uncorrelated sources of noise present in the two signals; Bendat and Piersol 1986). Measurements were made at an air temperature of $25 \pm 2 \text{ }^\circ\text{C}$.

We adjusted the amplitude of the random signal at the insect's feet to the minimum necessary to yield high coherence values. Although this varied between insects, the amplitudes used were within the range of amplitudes of the insects' own signals. Peak velocity amplitudes for signals recorded with the laser within a few cm of *U. crassicornis* adults on a hostplant stem were up to 0.6 mm s^{-1} for female signals and 2.1 mm s^{-1} for male advertisement signals (R.B. Cocroft, unpublished observations). RMS values from relatively constant-amplitude sections of the signals ranged from 0.2 mm s^{-1} (female signals) to 1.3 mm s^{-1} (male signals). RMS values for the random noise signal recorded on the dowel near the insect's tarsus ranged from 0.1 mm s^{-1} to 0.3 mm s^{-1} . We saw no evidence of non-linearities over this amplitude range in the mechanical response of the insects; the responses measured on all insects were qualitatively similar, and the high coherence values indicate that non-linearities did not play a major role.

Characterizing the mechanical response of the insect's body

Our goal in this study was to characterize the mechanical response of an intact, unrestrained insect on an appropriate substrate. An insect's body can be viewed as a system with interacting parts: head, thorax, abdomen, legs and wings. Our focus in this study was on the response properties of the thorax. Movements of the thorax relative to the stem will influence the movement of the legs, which contain the primary receptors for substrate vibration in most insects (Kalmring 1985). As in many membracid treehoppers, in *U. crassicornis* the exoskeletal roof of the first thoracic segment – the pronotum – extends over much of the body. We measured points along this rigid prothoracic extension as an index of the response of the thorax. For some individuals we also measured points on one middle leg, both on the tarsus where it attaches to the substrate and on the femur. Below we discuss the implications of the unusual morphology of this insect for the interpretation of our results.

For each individual, we measured seven points using the laser: four points on the insect, from front to back along the midline of the pronotum (Fig. 4); and three on the dowel, one directly in front of the insect, one directly behind, and one beside the tarsus of the middle leg. The points measured on the substrate allowed us to normalize the response of the insect's body relative to the substrate and to monitor the performance of the simulator (e.g., see Fig. 3). Each insect was placed in the center of the vertical dowel segment, facing up. For each point measured with the laser, the data collection program first generated a signal 'propagating' from front to back of the insect. Ten time samples (2048 points each, sampling rate 16 kHz) were obtained. The program then generated a signal 'propagating' in the opposite direction and again calculated the mean transfer function. The laser dot was then moved to the next point on the insect's body. If the insect changed its position or posture before all four points along the body were sampled, a new set of samples was obtained for the four points.

To characterize the motion of the insect's body relative to the vibrating substrate, we calculated transfer functions (see Bendat and Piersol 1986) between the signal measured on the substrate and the signal measured on the insect. This approach yields the

same results as, for example, driving the substrate with a sine wave that sweeps through the frequency range of interest; measuring the peak amplitude of the time waveform at each frequency, both on the substrate and on the insect; and taking the ratio of those amplitudes. A transfer function provides both the magnitude and the phase response, but in this report we focus only on the magnitude.

For each point measured with the laser, the time domain data were recorded simultaneously for the laser signal and the (a.c. coupled) input signal to the upper actuator. The data collection program then calculated a mean transfer function between the two signals. The transfer function was computed using the cross spectrum between the laser signal and the upper actuator signal, divided by the autospectrum of the upper actuator signal (Bendat and Piersol 1986). By processing each laser measurement in this manner we use a consistent reference signal (the upper actuator signal), allowing us to compute the transfer function between pairs of non-simultaneous laser measurements by taking the ratio of the individual transfer functions. After all the points were sampled, each transfer function obtained on the insect was normalized relative to the transfer function measured on the dowel near the insect's middle leg. Our results thus show how the insect's body moved relative to the substrate. Furthermore, for each point measured on the insect, our measurements show the response to signals propagating in both directions.

For a subset of insects, we rotated the simulator horizontally 45° from the plane of the laser to allow measurements along the right middle leg, which otherwise was blocked by the pronotum. The first and third pairs of legs remained partially blocked. Measured individuals were marked with reflective paint on the tarsus and femur (Fig. 4). Three points were sampled: on the dowel adjacent to the tarsus (where the leg attaches to the substrate), on the tarsus, and on the femur. Note that our laser measurements on the body are in the plane parallel to the driving motion of the actuators. Rotating the simulator relative to the laser yields measurements of this motion that are reduced by a factor proportional to the cosine of the angle of rotation. However, when we normalize the leg measurements relative to measurements on the substrate made at the same angle, this multiplicative factor cancels out. The measurements on the leg are thus directly comparable to the measurements on the body.

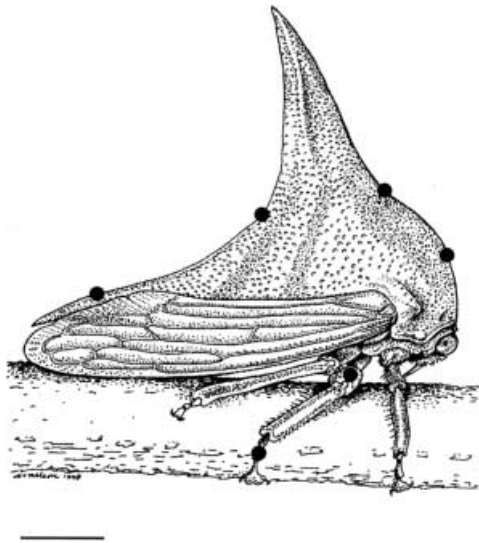


Fig. 4 Female *U. crassicornis* in a typical position on a host plant stem. Black dots show locations of laser recordings: four points along the midline of the pronotum (anterior, mid-anterior, mid-posterior and posterior), and two points on the tarsus and femur of the right middle leg. At each point, data were acquired with signals propagating in both directions, and with the laser measuring motion parallel to the dorso-ventral plane of the insect. Scale bar equals 2 mm

Results

Vibrational signals

We measured the propagation velocity of signals produced by adult females ($n = 2$, 5 signals each) on a host plant stem. The female signals had a broad frequency spectrum (Fig. 5b), allowing for measurements from 100 to 2500 Hz. Clear recordings of the signals were obtained at both the near and far accelerometers, as reflected in the high coherence values obtained between the two (Fig. 5c). The measured phase propagation velocity (Fig. 5a) increases with the square root of frequency, as expected for bending waves (Fung 1965). The frequency-velocity relationship is similar to that measured previously in a different host plant (Tieu 1996) and used in designing the bending wave simulator. The measured velocities are of the same order of magnitude as the group velocities of mechanically induced bending waves measured by Michelsen et al. (1982), Keuper and Kühne (1983), and Barth (1998). The vibrational signals of *U. crassicornis* are evidently transmitted along plant stems in the form of bending waves.

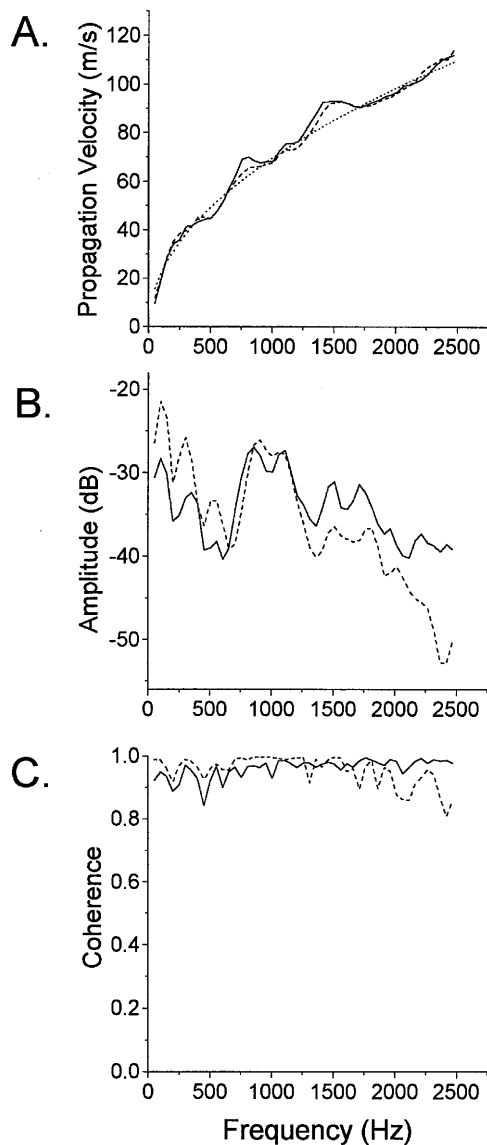


Fig. 5A–C Propagation velocity measurements of signals produced by female *U. crassicornis* on a host plant stem. **A** Solid and dashed lines are mean velocity measurements for signals of two females ($n = 5$ signals each); dotted curve (fitted by eye) is proportional to the square root of frequency. **B** Mean amplitude spectra of signals of the two females. **C** Mean coherence between signals recorded at near and far accelerometers for each female ($n = 5$ signal pairs each)

The substrate-insect transfer function and the influence of direction

Measurements of the response of the insects ($n = 29$ females) on the stem simulator (Fig. 1) are shown in Fig. 6. If the amplitude spectrum of the random noise signal was unchanged as it passed from the substrate into the insects' body, the transfer function magnitude would be a flat line at 0 dB. However, in all four locations along the pronotum, and with both stimulus directions, the amplitude spectrum of the signal measured on the insect differed substantially from that measured on the substrate. At lower frequencies, from 150 Hz to

200 Hz, the vibration amplitude was about equal to that of the substrate (150 Hz was the lowest frequency for which we could obtain high coherence between the output signal and the signal recorded on the insect). There was a resonant peak (± 5 dB to ± 10 dB) between 500 Hz and 700 Hz at each location on the pronotum, for at least one stimulus direction. At higher frequencies, from 1 kHz to 4 kHz, there was increasing attenuation (-4 to -14 dB at the highest frequencies), with a suggestion of a second, lower-amplitude peak at about 1500 Hz.

The mechanical response of the body relative to the substrate varied with stimulus direction, especially between 300 Hz and 3000 Hz (Fig. 6). Differences were greatest at the two ends of the pronotum, where the mean amplitude near the resonant peak differed by 8–10 dB. These differences are due solely to the direction of the stimulus; the stimulus amplitude spectrum and the insect's position and posture were identical. This pattern was consistent in all 29 individuals examined. Although the relationship in Fig. 6 is based on measurements of the velocity component of the signals, because the signals were normalized relative to the substrate the same relationship would hold for displacement or acceleration measurements. Figure 7 shows the relative motion of the anterior and posterior pronotum. When the wave originated in front of the insect, the two locations had a similar amplitude spectrum, although below 2500 Hz the amplitude at the anterior end was greater. However, when the wave originated behind the insect, the amplitude was higher at the posterior pronotum for all frequencies.

We also measured movement of the middle leg relative to the substrate ($n = 9$ females). Movement of the tarsus (the point of attachment to the stem) closely follows that of the substrate (Fig. 8a). However, movement of the femur differs from that of the substrate, showing a pattern of resonance and attenuation similar to that of the thorax (Fig. 8b). The femur also responds differently depending on stimulus direction.

Further analysis of the data from four arbitrarily chosen individuals revealed the presence of two distinct modes of vibration in the insect's body along its long axis (Fig. 9). In the translational mode, the front and back move in phase with each other. In the rotational mode, the front and back move in antiphase. At most frequencies both modes are evident, but at some frequencies the movement is almost purely translational (lower frequencies) or rotational (higher frequencies).

Discussion

We show for the first time that the direction of wave propagation influences the mechanical response of an insect's body to substrate vibration. Our study was motivated by the consideration that a biomechanical directionality, if it exists, could provide a source of directional information for insects that use substrate

Fig. 6A–D The influence of direction on the mechanical response of the insect’s body. Panels **A–D** show mean and 95% confidence interval for transfer functions at four points along the pronotum ($n = 29$ females). The y -axis represents the amplitude of the signal recorded on the insect relative to that recorded on the substrate, expressed on a decibel scale. *Solid line and light grey interval*: signal originating in front of the insect; *dotted line and dark grey interval*: signal originating behind the insect

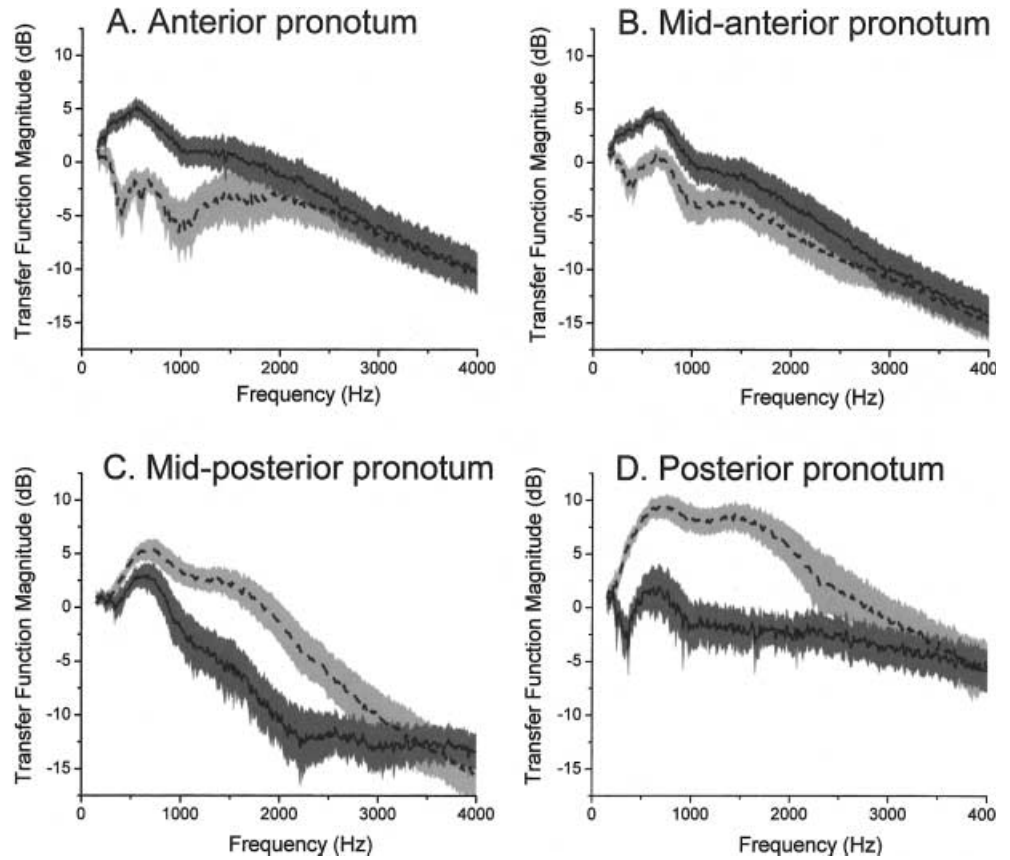


Fig. 7A, B Comparison of the amplitude of the signals recorded on the anterior and posterior pronotum, normalized with respect to the signal recorded on the substrate (conventions as in Fig. 6; $n = 29$ females). In **A** the signal in the substrate originates in front of the insect, in **B** behind the insect

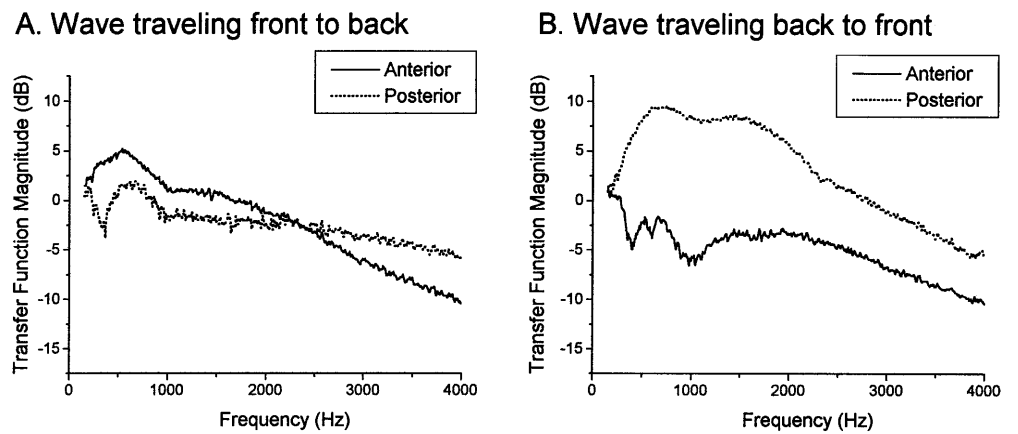
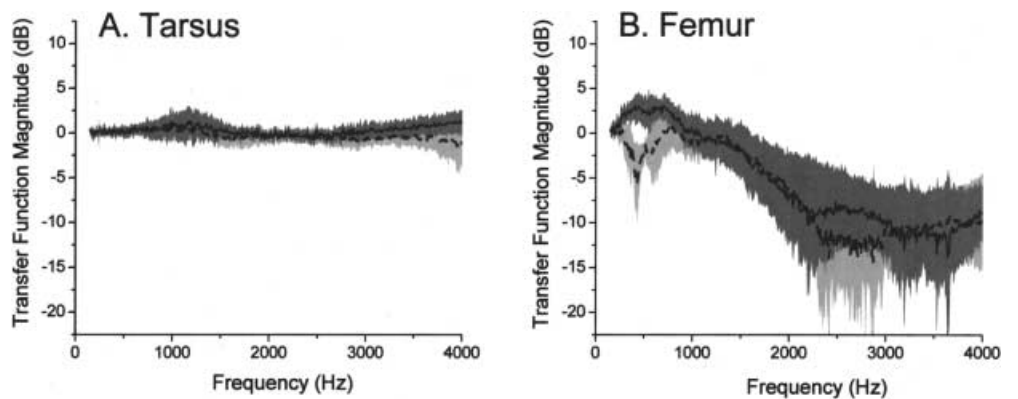


Fig. 8A, B The influence of direction on the mechanical response of the tarsus (A) and femur (B) of the middle leg (conventions as in Fig. 6; $n = 9$ females)



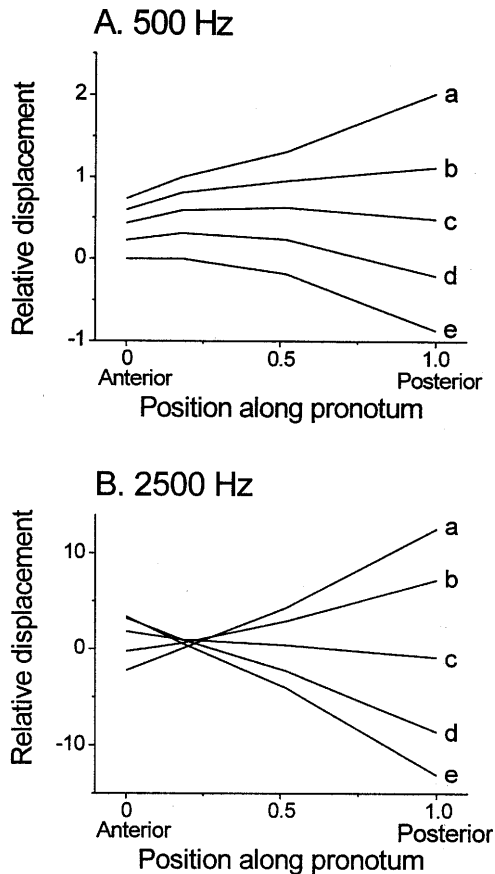


Fig. 9A, B Modes of vibration measured on the pronotum, in an example from one individual. Each line represents motion of the pronotum as a whole, at sequential time intervals (*a-e*) equally spaced through one-half of a cycle. **A** Translational mode (seen at 500 Hz), in which anterior and posterior points move in phase. **B** Rotational mode (seen at 2500 Hz), in which anterior and posterior points move out of phase. The posterior pronotum moves with greater amplitude in both cases

vibrations in social and ecological interactions (Markl 1983; Gogala 1985; Henry 1994; Meyhöfer et al. 1997; Stewart 1997). The study species was the treehopper *U. crassicornis*, which communicates using vibrational signals transmitted through plant stems (Cocroft 1996, 1999). Our experimental protocol allowed us to characterize the mechanical response of free-standing, stationary insects while varying the direction of travel of a random noise vibration.

The body of an insect or other terrestrial arthropod is analogous to a mass on a set of springs (Aicher et al. 1983; Tieu 1996; Dierkes and Barth 1995). In the treehopper we studied, as well as in a spider (Dierkes and Barth 1995) and a fiddler crab (Aicher et al. 1983), there was a resonant peak in the response of the body to substrate vibration, with attenuation at higher frequencies. This pattern is characteristic of a mass-and-spring system (Rao 1995). In an insect, the system is further complicated by subdivision of the mass into a thorax and a flexible abdomen, which may have its own modes of vibration (Tieu 1996; R.B. Cocroft and R.N. Miles,

unpublished data). Furthermore, the magnitude of the resonance will depend on such factors as the stiffness and damping of the legs. As a result, the response properties of an oscillating system comprising an arthropod's body and legs are likely to vary within individuals when posture changes (Aicher et al. 1983; Dierkes and Barth 1995) and from species to species. It is possible that such differences may explain why investigators found no detectable resonant peak in the legs of a cricket (Dambach 1972) and a honeybee (Rohrseitz and Kilpinen 1997); i.e., these may represent highly damped systems.

In the parasitoid fly *O. ochracea*, in which females use airborne sound to locate calling male crickets, the two ears span only a tiny fraction of the wavelength of the sound (Miles et al. 1995). However, the ears are mechanically coupled, and the interaction of two modes of vibration (one translational, one rotational) converts a minuscule phase difference into a relatively large, directional amplitude difference at the two ears. The directionality we observed in the mechanical response of the body of *U. crassicornis* to substrate vibration may be analogous. We observed both a translational mode and a rotational mode in the movement of the insect's body relative to the substrate, and it is likely that the interaction of these modes is responsible for the mechanical directionality of the body. In addition to the two modes of vibration along the body's long axis, preliminary observations (R.B. Cocroft and R.N. Miles, unpublished data), suggest there is at least occasionally a lateral rotational mode (i.e., a side-to-side 'rocking') which may contribute to the overall mechanical response of the body to substrate vibration.

Membracid treehoppers have an expanded pronotum, which in *U. crassicornis* covers much of the body. How much of the mechanical directionality we documented is due to this unusual structure? Possession of an enlarged pronotum is apparently not necessary for generating a basic pattern of resonance and attenuation, since similar patterns occur in the mechanical response to vibration in two other arthropods, a crab (Aicher et al. 1983) and a spider (Dierkes and Barth 1995). Furthermore, preliminary data using individuals with most of the pronotum removed ($n = 3$) revealed that mechanical directionality was very similar to those in intact insects (R.B. Cocroft and R.N. Miles unpublished data). We suggest that the main influence of the expanded pronotum is likely to be in its contribution to the mass of the body. Accordingly, we see no reason why the results recorded with *U. crassicornis* should not be encountered in other insects with different morphologies.

How might *U. crassicornis* detect the directional information present in the mechanical response of the body? Although there are sensory structures in the pronotum (Wood and Morris 1974), information present in the response of the legs is more likely to be accessible to vibration receptors. Membracids likely use both subgenual and chordotonal organs in vibration perception, as do some other Hemiptera (Cokl 1983).

The responses we measured on the pronotum may not translate directly to responses elsewhere on the body (e.g., the amplitude of movement on the posterior extension of the pronotum may be higher than that in the legs). However, measurements of the middle leg showed that resonance, attenuation, and the effect of stimulus direction can be detected on the femur. Furthermore, preliminary data from individuals with most of the pronotum removed ($n = 3$), which allowed measurements on all of legs on one side, showed that the transfer function measured on the thorax was similar to that on the femur of the fore and hind legs as well. The effect of direction was different on the fore and hind legs (as it was on the anterior and posterior pronotum; see Fig. 7). It is possible then, as the pronotal measurements (this study) and leg measurements (R.N. Miles and R.B. Cocroft, unpublished data) both suggest, that the responses at the front and back of the body are relatively similar when the stimulus originates in front of the insect, but different when the stimulus originates behind the insect. If so, a comparison of signal amplitude between receptors in the front and back legs might provide a simple mechanism of vibration localization.

Mechanisms of localization in two dimensions have been investigated in several arthropods that use surface waves on sand or water to locate prey. Desert scorpions (Brownell and Farley 1979), wandering spiders (Hergenröder and Barth 1983), water striders (Murphey 1971) and backswimmers (Wiese 1974) can use differences in arrival times at receptors located in different legs to orient to a vibration source. There is also some evidence for the use of amplitude gradients in localization (Rupprecht 1968; Brownell and Farley 1979; Hergenröder and Barth 1983; Latimer and Schatral 1983; Cokl et al. 1985). In contrast, mate-locating male deathwatch beetles and stoneflies on a two-dimensional surface did not show clear directionality in their turning responses to female vibrational signals (Abbott and Stewart 1993; Goulson et al. 1994). However, as the authors noted, the surfaces used in those experiments may present a more challenging localization task than do the insects' natural substrates. Several studies have also investigated whether insects can localize a vibration source when presented with two stems at a branching point (Latimer and Schatral 1983; Ota and Cokl 1991; Roces et al. 1993; Pfannenstiel et al. 1995). Although these studies have found a preference for the stem containing the vibration source, the mechanism of this choice has not been investigated. Potential cues include arrival time and amplitude differences between receptors monitoring different stems (Morris 1980; Latimer and Schatral 1983), although the latter may not always be reliable given that side branches and leaves can vibrate at a higher amplitude than the stem on which the signal originates (Keuper and Kühne 1983).

A rodlike structure such as a plant stem provides a one-dimensional localization problem for an insect oriented lengthwise along the structure. Although this context is simpler than the two-dimensional one (the

choice is between 0° and 180° rather than among angles varying continuously from 0° to 360°), there have been relatively few studies of localization along stems. In an investigation of vibration-sensitive neurons in locusts, Cokl et al. (1985) showed that the time and amplitude differences resulting from propagation of bending waves in plant stems should be sufficient to provide directionality in these relatively large insects (distance between fore- and hind-legs 5 cm). We are not aware of studies of directional behavior in smaller species, in which arrival time differences between the legs may be one or two orders of magnitude smaller than in locusts. Arrival time differences between front and hind legs of *U. crassicornis*, which are separated by about 5 mm, will be relatively small. For example, given the group velocity of the bending waves we measured (twice the phase velocity and representing the propagation velocity of the envelope of a transient signal; Michelsen et al. 1982; Markl 1983), the difference in arrival time between the two legs will be 55 μ s at 500 Hz and 37 μ s at 1000 Hz.

The propagation of bending waves within the complex geometry of a plant can impose constraints on localization along a stem. First, amplitude may not decrease monotonically with distance from the source (Michelsen et al. 1982). Especially for localization of signals with a narrow frequency band, this could cause an insect using an amplitude gradient to orient to a local amplitude peak some distance from the vibration source. Second, reflected waves are prominent under some conditions (Michelsen et al. 1982; Keuper and Kühne 1983). Mechanisms of localization based on arrival time differences will only be reliable if there is a wave propagating in one direction along the stem. Our measurements of bending waves in a leafy plant stem show that one-directional wave propagation can occur with insect signals in natural substrates. But given the existence of environmental constraints on vibrotaxis in some contexts, we might also expect behavioral rules that come into play when direct localization based on vibrational information is not possible. For example, male leafhoppers (*Graminella nigrifrons*) orient toward light while searching for females (Hunt and Nault 1991). Goulson et al. (1994) proposed two other alternatives to vibrotaxis: triangulation, in which an individual samples multiple points before orienting; and a searching rule in which the individual makes large turning angles when the amplitude of the signal drops below a certain threshold.

Behavioral directionality and the neural basis of vibration perception in *U. crassicornis* have not yet been investigated. However, there are contexts in which the ability to localize a signal would be adaptive. For example, both mate location (R.B. Cocroft, unpublished data) and maternal defense of offspring (Cocroft 1996) are mediated by vibrational signals. We might expect a range of localization mechanisms in a small insect using plant-borne vibrations, including mechanisms like those proposed for other arthropods (Brownell and Farley 1979; Hergenröder and Barth 1983) and behavioral

rules-of-thumb (Hunt and Nault 1991; Goulson et al. 1994). Based on the results of this study, we suggest that directional information present in the mechanical response of the body may provide another localization mechanism, which could be especially important for species that encounter small time and amplitude differences between receptors.

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